

At page 121, line 12, please delete "C (asp)" all three occurrences and substitute therefor --C (ala)--.

At page 122, line 11, please delete "C (asp)" all three occurrences and substitute therefor --C (ala)--.

IN THE CLAIMS:

Please cancel claims 1-62 and add new claims 63-147 as follows consistent with the restriction requirement mailed on December 10, 1997 in the above identified parent application, without prejudice.

Please add new claims 63-147 as follows:

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- 1 --63. An isolated infectious recombinant respiratory syncytial virus (RSV)
2 comprising a RSV genome or antigenome, a major nucleocapsid (N) protein, a nucleocapsid
3 phosphoprotein (P), a large polymerase protein (L), and a RNA polymerase elongation factor,
4 wherein a modification is introduced within the genome or antigenome comprising a deletion,
5 insertion, substitution, rearrangement, or nucleotide modification of a cis-acting regulatory
6 sequence within the recombinant RSV genome or antigenome.
- 1 64. The recombinant RSV of claim 63, wherein the cis-acting regulatory
2 sequence is a gene-start (GS) signal or a (GE) signal.
- 1 65. The recombinant RSV of claim 64, wherein a GS or GE signal is deleted
2 or inserted in the genome or antigenome.
- 1 66. The recombinant RSV of claim 64, wherein a GS or GE signal is
2 substituted in the genome or antigenome by a heterologous GS or GE sequence.
- 1 67. The recombinant RSV of claim 66, wherein the heterologous GS or GE
2 sequence is of a different RSV gene.

1 68. The recombinant RSV of claim 67, wherein a GE signal of the RSV NS1
2 or NS2 gene is replaced by a corresponding GE sequence of the RSV N gene.

1 69. The recombinant RSV of claim 66, wherein the heterologous GS or GE
2 sequence is of a heterologous negative stranded virus.

1 70. The recombinant RSV of claim 69, wherein the heterologous GS or GE
2 sequence is of a human RSV A or RSV B subgroup.

1 71. The recombinant RSV of claim 69, wherein the heterologous GS or GE
2 sequence is of a non-human RSV.

1 72. The recombinant RSV of claim 70, wherein the heterologous GS or GE
2 sequence is of a bovine RSV.

1 73. The recombinant RSV of claim 70, wherein the heterologous GS or GE
2 sequence is of a parainfluenza virus (PIV).

1 74. The recombinant RSV of claim 73, wherein the heterologous GS or GE
2 sequence is of a PIV3 virus.

1 75. The recombinant RSV of claim 64, wherein a nucleotide sequence of a
2 gene-start (GS) or gene-end (GE) signal is altered in the genome or antigenome.

1 76. The recombinant RSV of claim 64, wherein a gene-start (GS) or gene-
2 end (GE) signal is rearranged by changing a position of the (GS) or gene-end (GE) signal in
3 the recombinant genome or antigenome.

1 77. The recombinant RSV of claim 63, wherein the cis-acting regulatory
2 sequence occurs within a 3' leader, 5' trailer or intergenic region of the RSV genome or
3 antigenome.

1 78. The recombinant RSV of claim 77, wherein the cis-acting regulatory
2 sequence is a RSV promoter element.

1 79. The recombinant RSV of claim 78, wherein a promoter element is
2 deleted or inserted in the genome or antigenome.

1 80. The recombinant RSV of claim 78, wherein a promoter element is
2 substituted in the genome or antigenome by a heterologous promoter element.

1 81. The recombinant RSV of claim 80, wherein the heterologous promoter
2 element is of a different RSV gene.

1 82. The recombinant RSV of claim 80, wherein the heterologous promoter
2 element is of a heterologous negative stranded virus.

1 83. The recombinant RSV of claim 82, wherein the heterologous promoter
2 element is of a human RSV A or RSV B subgroup.

1 84. The recombinant RSV of claim 82, wherein the heterologous promoter
2 element is of a non-human RSV.

1 85. The recombinant RSV of claim 84, wherein the heterologous GS or GE
2 sequence is of a bovine RSV.

1 86. The recombinant RSV of claim 82, wherein the heterologous promoter
2 element is of a parainfluenza virus (PIV).

1 87. The recombinant RSV of claim 86, wherein the heterologous promoter
2 element is of a PIV3 virus.

1 88. The recombinant RSV of claim 78, wherein a nucleotide sequence of a
2 promoter element is altered in the genome or antigenome.

1 89. The recombinant RSV of claim 78, wherein a promoter element is
2 rearranged by changing a position of the promoter element in the recombinant genome or
3 antigenome.

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1 90. The recombinant RSV of claim 63, wherein a further modification is
2 introduced within the recombinant genome or antigenome comprising a partial or complete
3 gene deletion, a change in gene position, or one or more nucleotide change(s) that modulate
4 expression of a selected gene.

1 91. The recombinant RSV of claim 90, wherein a RSV gene is deleted in
2 whole or in part.

1 92. The recombinant RSV of claim 91, wherein a SH, NS1, NS2, or G gene
2 is deleted in whole or in part.

1 93. The recombinant RSV of claim 90, wherein expression of a selected
2 RSV gene is reduced or ablated by introduction of one or more translation termination codons.

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1 94. The recombinant RSV of claim 90, wherein expression of a selected
2 RSV gene is reduced or ablated by introduction of multiple translation termination codons.

1 95. The recombinant RSV of claim 90, wherein expression of a selected
2 RSV gene is reduced or ablated by introduction of a frame shift mutation in the gene.

1 96. The recombinant RSV of claim 90, wherein expression of a selected
2 RSV gene is modulated by introduction, modification or ablation of a translational start site
3 within the gene.

1 97. The recombinant RSV of claim 90, wherein a position of one or more
2 gene(s) in the recombinant genome or antigenome is altered relative to a RSV promoter.

1 98. The recombinant RSV of claim 97, wherein said position of said one or
2 more gene(s) is changed to a more promoter-proximal or promoter-distal location by deletion
3 or insertion of a coding or non-coding polynucleotide sequence within the recombinant
4 genome or antigenome upstream of said one or more gene(s).

1 99. The recombinant RSV of claim 97, wherein positions of multiple genes
2 in the recombinant genome or antigenome are altered by changing their relative gene order.

1 100. The recombinant RSV of claim 63, wherein the recombinant genome or
2 antigenome is further modified to incorporate one or more attenuating mutation(s) present in
3 one or more biologically derived mutant human RSV strain(s).

1 101. The recombinant RSV of claim 100, wherein the recombinant genome
2 or antigenome is further modified to incorporate at least one and up to a full complement of
3 attenuating mutations present within a panel of biologically derived mutant human RSV
4 strains, said panel comprising cpts RSV 248 9ATCC VR 2450), cpts RSV 248/404 (ATCC VR
5 2454), cpts RSV 248/955 (ATCC VR 2453), cpts RSV 530 (ATCC VR 2452), cpts RSV
6 530/1009 (ATCC VR 2451), cpts RSV 530/1030 (ATCC VR 2455), RSV B-1 cp52/2B5
7 (ATCC VR 2542), and RSV B-1 cp-23 (ATCC VR 2579).

1 102. The recombinant RSV of claim 100, wherein the recombinant genome
2 or antigenome is further modified to incorporate at least one and up to a full complement of
3 attenuating mutations specifying an amino acid substitution at Val267 in the RSV N gene,
4 Glu218 and/or Thr523 in the RSV F gene, Cys319 Phe 521, Gln831, Met1169, Tyr1321 and/or
5 His 1690 in the RSV polymerase gene L, and a nucleotide substitution in the gene-start
6 sequence of gene M2.

1 103. The recombinant RSV of claim 100, wherein the recombinant genome
2 or antigenome incorporates at least two attenuating mutations.

1 104. The recombinant RSV of claim 63, wherein the recombinant genome or
2 antigenome comprises a partial or complete human RSV genome or antigenome of one RSV
3 subgroup or strain combined with a heterologous gene or gene segment from a different,
4 human or non-human RSV subgroup or strain to form a chimeric genome or antigenome.

1 105. The recombinant RSV of claim 104, wherein the heterologous gene or
2 gene segment is from a human RSV subgroup A, human RSV subgroup B, bovine RSV, or
3 murine RSV.

1 106. The recombinant RSV of claim 104, wherein the chimeric genome or
2 antigenome comprises a partial or complete human RSV A subgroup genome or antigenome

3 combined with a heterologous gene or gene segment encoding a RSV F, G or SH glycoprotein
4 or a cytoplasmic domain, transmembrane domain, ectodomain or immunogenic epitope thereof
5 from a human RSV B subgroup virus.

1 107. The chimeric RSV of claim 106, wherein both human RSV B subgroup
2 glycoprotein genes F and G are substituted to replace counterpart F and G glycoprotein genes
3 in a partial RSV A genome or antigenome.

1 108. The recombinant RSV of claim 106, wherein the chimeric genome or
2 antigenome comprises a partial or complete human RSV B subgroup genome or antigenome
3 combined with a heterologous gene or gene segment from a human RSV A subgroup virus.

1 109. The recombinant RSV of claim 104, wherein the chimeric genome or
2 antigenome comprises a partial or complete RSV background genome or antigenome of a
3 human or bovine RSV combined with a heterologous gene or genome segment of a different
4 RSV to form a human-bovine chimeric RSV genome or antigenome.

1 110. The recombinant RSV of claim 63, wherein the recombinant genome or
2 antigenome incorporates a heterologous gene or genome segment from parainfluenza virus
3 (PIV).

1 111. The recombinant RSV of claim 110, wherein the gene or genome
2 segment encodes a PIV HN or F glycoprotein or immunogenic domain or epitope thereof.

1 112. The recombinant RSV of claim 110, wherein the genome segment
2 encodes one or more immunogenic protein(s), protein domain(s) or epitope(s) HPIV1, HPIV2,
3 and/or HPIV3.

1 113. The recombinant RSV of claim 63, wherein the recombinant genome or
2 antigenome is further modified to encode a non-RSV molecule selected from a cytokine, a T-
3 helper epitope, or a protein of a microbial pathogen capable of eliciting a protective immune
4 response in a mammalian host.

1 114. The recombinant RSV of claim 63 which is a virus.

1 115. The recombinant RSV of claim 63 which is a subviral particle.

1 116. The recombinant RSV of claim 63, formulated in a dose of 10³ to 10⁶
2 PFU of attenuated virus.

1 117. A method for stimulating the immune system of an individual to induce
2 protection against respiratory syncytial virus, which comprises administering to the individual
3 an immunologically sufficient amount of the isolated attenuated recombinant RSV of claim 1.

1 118. The method of claim 117, wherein the recombinant virus is administered
2 in a dose of 10³ to 10⁶ PFU of the attenuated RSV.

1 119. The method of claim 117, wherein the recombinant virus is administered
2 to the upper respiratory tract.

1 120. The method of claim 119, wherein the recombinant virus is administered
2 by spray, droplet or aerosol.

1 121. The method of claim 117, wherein the recombinant virus is administered
2 to an individual seronegative for antibodies to RSV or possessing transplacentally acquired
3 maternal antibodies to RSV.

1 122. A vaccine to induce protection against RSV, which comprises an
2 immunologically sufficient amount of the isolated attenuated recombinant RSV of claim 1 in a
3 physiologically acceptable carrier.

1 ~~123. The vaccine of claim 122, formulated in a dose of 10³ to 10⁶ PFU of the~~
2 ~~attenuated RSV.~~

1 ~~124. The vaccine of claim 122, formulated for administration to the upper~~
2 ~~respiratory tract by spray, droplet or aerosol.~~

1 125. The vaccine of claim 122, wherein the recombinant RSV elicits an
2 immune response against human RSV A, human RSV B, or both.

1 126. An expression vector comprising an isolated polynucleotide molecule
2 encoding a respiratory syncytial virus (RSV) genome or antigenome modified by a deletion,
3 insertion, substitution, rearrangement, or nucleotide modification of a cis-acting regulatory
4 sequence.

1 127. An isolated polynucleotide molecule comprising a respiratory syncytial
2 virus (RSV) genome or antigenome which is modified by a deletion, insertion, substitution,
3 rearrangement, or nucleotide modification of a cis-acting regulatory sequence.

1 128. The isolated polynucleotide molecule of claim 127, wherein the cis-
2 acting regulatory sequence is a gene-start (GS) signal or a (GE) signal.

1 129. The isolated polynucleotide molecule of claim 127, wherein the cis-
2 acting regulatory sequence occurs within a 3' leader, 5' trailer or intergenic region of the RSV
3 genome or antigenome.

1 130. The isolated polynucleotide molecule of claim 127, wherein the cis-
2 acting regulatory sequence is a RSV promoter element.

1 131. The isolated polynucleotide molecule of claim 127, wherein a further
2 modification is introduced within the recombinant genome or antigenome comprising a partial
3 or complete gene deletion, a change in gene position, or one or more nucleotide change(s) that
4 modulate expression of a selected gene.

1 132. The isolated polynucleotide molecule of claim 131, wherein a RSV gene
2 is deleted in whole or in part.

1 133. The isolated polynucleotide molecule of claim 127, wherein expression
2 of a selected RSV gene is reduced or ablated by introduction of one or more translation
3 termination codons in the recombinant genome or antigenome.

1 134. The isolated polynucleotide molecule of claim 127, wherein expression
2 of a selected RSV gene is reduced or ablated by introduction of a frame shift mutation in the
3 gene.

1 135. The isolated polynucleotide molecule of claim 127, wherein expression
2 of a selected RSV gene is modulated by introduction, modification or ablation of a
3 translational start site within the gene.

1 136. The isolated polynucleotide molecule of claim 127, wherein a position
2 of one or more gene(s) in the recombinant genome or antigenome is altered relative to a RSV
3 promoter.

1 137. The isolated polynucleotide molecule of claim 127, wherein the
2 recombinant genome or antigenome is further modified to incorporate one or more attenuating
3 mutation(s) present in one or more biologically derived mutant human RSV strain(s).

1 138. The isolated polynucleotide molecule of claim 137, wherein the
2 recombinant genome or antigenome is further modified to incorporate at least one and up to a
3 full complement of attenuating mutations specifying an amino acid substitution at Val267 in
4 the RSV N gene, Glu218 and/or Thr523 in the RSV F gene, Cys319 Phe 521, Gln831,
5 Met1169, Tyr1321 and/or His 1690 in the RSV polymerase gene L, and a nucleotide
6 substitution in the gene-start sequence of gene M2.

1 139. The isolated polynucleotide molecule of claim 27, wherein the
2 recombinant genome or antigenome comprises a partial or complete human RSV genome or
3 antigenome of one RSV subgroup or strain combined with a heterologous gene or gene
4 segment from a different, human or non-human RSV subgroup or strain to form a chimeric
5 genome or antigenome.

1 140. The isolated polynucleotide molecule of claim 139, wherein the
2 heterologous gene or gene segment is from a human RSV subgroup A, human RSV subgroup
3 B, bovine RSV, or murine RSV.

1 141. The isolated polynucleotide molecule of claim 140, wherein the
2 chimeric genome or antigenome comprises a partial or complete human RSV A subgroup
3 genome or antigenome combined with a heterologous gene or gene segment encoding a RSV

4 F, G or SH glycoprotein or a cytoplasmic domain, transmembrane domain, ectodomain or
5 immunogenic epitope thereof from a human RSV B subgroup virus.

1 142. The isolated polynucleotide molecule of claim 141, wherein both human
2 RSV B subgroup glycoprotein genes F and G are substituted to replace counterpart F and G
3 glycoprotein genes in a partial RSV A genome or antigenome.

1 143. The recombinant RSV of claim 127, wherein the recombinant genome
2 or antigenome comprises a partial or complete RSV background genome or antigenome of a
3 human or bovine RSV combined with a heterologous gene or genome segment of a different
4 RSV to form a human-bovine chimeric RSV genome or antigenome.

1 144. The isolated polynucleotide molecule of claim 127, wherein the
2 recombinant genome or antigenome incorporates a heterologous gene or genome segment from
3 parainfluenza virus (PIV).

1 145. The isolated polynucleotide molecule of claim 27, wherein the
2 recombinant genome or antigenome is further modified to encode a non-RSV molecule
3 selected from a cytokine, a T-helper epitope, or a protein of a microbial pathogen capable of
4 eliciting a protective immune response in a mammalian host.

1 146. A method for producing an infectious respiratory syncytial virus (RSV)
2 particle from one or more isolated polynucleotide molecules encoding said RSV, comprising:

3 expressing in a cell or cell-free lysate an expression vector comprising an
4 isolated polynucleotide comprising a recombinant RSV genome or antigenome which is
5 modified by a deletion, insertion, substitution, rearrangement, or nucleotide modification of a
6 cis-acting regulatory sequence.

1 147. The method of claim 146, wherein the recombinant RSV genome or
2 antigenome and the N, P, L and RNA polymerase-elongation factor proteins are expressed by
3 two or more different expression vectors.